CLAIMS:

1. A putative protective antigen against a Mycoplasma, prepared by a method including

providing

a sample of a Mycoplasma;

an antibody probe including at least one antibody against a Mycoplasma produced by a method including;

providing a biological sample taken a short time after an immune animal has been challenged with a Mycoplasma or Mycoplasma extract taken from the infection site or an area of a lesion or an area close to the infection site or lesion;

isolating cells from the biological sample;

culturing cells in vitro in a suitable culture medium; and

harvesting antibodies produced from said cells;

probing the Mycoplasma sample with the antibody probe to detect at least one antigen; and

isolating the antigen detected.

2. A putative protective antigen according to claim 1 wherein the Mycoplasma is Mycoplasma hyopneumoniae.

3. A putative protective antigen against Mycoplasma hyopneumoniae, or related infections, selected from the group of antigens having approximate molecular weights of 110-114, 90-94, 72-75, 50-54, 52-54 and 46-48 kilodaltons (kD), as herein described, mutants, derivatives and fragments thereof.

4. A putative protective antigen according to claim 3 which is a surface protein.

5. A putative protective antigen according to claim 3 or 4 which is a surface lipo-protein or membrane protein.

6. A putative protective antigen according to any one of claims 3-5 having approximate molecular weight of 110-114, 90-94, 74, 62, 52 and 48 kD.

7. A putative protective antigen according to claim 3 wherein the antigen in the 72-75 kD region contains the following N-terminal amino acid sequence:

AGXLQKNSLLEEVWYLAL

8. A putative protective antigen according to claim 7 further including one or more of the following N-terminal amino acid sequences:

AKNFDFAPSIQGYKKIAHEL

NLKPEQILQLLG

LLKAEXNKXIEEINTXLDN

9. A putative protective antigen according to claim 3 wherein the antigen in the 60-64 kD region contains the following N-terminal amino acid sequence:

MKLAKLLKGFX(N/L)(M/V)IK

ADP(F/I)(R/E)Y(V/A)PQG(Q/A)X(M/N)VG

10. A putative protective antigen according to claim 3 wherein the antigen in the 52-54 kD region contains the following N-terminal amino acid sequence:

AGXWAKETTKEEKS

11. A putative protective antigen according to claim 10 further including one or more of the following N-terminal amino sequences:

AWVTADGTVN

AIVTADGTVNDNKPNQWVRKY

12 A putative protective antigen according to claim 3 wherein the antigen in the 46-48 kD region contains the following N-terminal amino acid sequence:

AGXGQTESGSTSDSKPQAETLKHKV

13. A putative protective antigen according to claim 12 further including one or more of the following internal amino acid sequences:

TIYKPDKVLGKVAVEVLRVLIAKKNKASR

AEQAITKLKLEGFDTQ

KNSQNKIIDLSPEG

14. An isolated nucleic acid fragment encoding a putative protective antigen against Mycoplasma hyopneumoniae or related infections, said nucleic acid fragment including the following sequence, mutants, derivatives, recombinants and fragments thereof:

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
ATGAAAAAAA	TGCCACTATA	CCAGAGGAAA	GAGCAGTATA	TAAAATAATT	50
AAAATTACAT	TTTCTTCATT	TGCGCCAGAA	TTTTTAAGAA	TTAGTACATT	100
AAAAAGTAGA	ACAAAAGTTA	TTAATGTAAA	CATTAGCGCA	ATCCTTAAGA	150
AAAAATTAAA	AGTTTTATCT	ATTTTTTTTA	ATCGAAATCC	AACCAGGCAT	200
AAATCTTTGT	CAGTATTTAT	CAAGTCGGTA	TTTTTTCATT	ATTTCTACTA	250
AAATATTATT	TGAATTTGCA	TTTTCCATAA	TCTAAAATTT	TACATTTTTT	300
TATAACAATT	TTTAAAAATT	ACTCTTTAAT	TTATAGTATT	TTTTTATTTT	350
TTAGTCTAAA	TTATAAAATT	ATCTTGAATT	TTATTTGAAT	TTTTATAATT	400
TAGTACTAAA	AAATACAAAT	ATTTTTTCCT	ATTCTAAGAA	AAATTCATTT	450
TTTAAAAAAA	ATTGATTTTT	ATAGTATAAT	TTGTTTGTAT	AATTGAATTA	500
ACTTGATTTG	AAAGGGAACA	AAATGAAAAA	AATGCTTAGA	AAAAAATTCT	550
TGTATTCATC	AGCTATTTAT	GCAACTTCGC	TTGCATCAAT	TATTGCATTT	600
GTTGCAGCAG	GTTGTGGACA	GACAGAATCA	GGTTCAACTT	CTGATTCTAA	650
ACCACAAGCC	GAGACGCTAA	AACATAAAGT	AAGTAATGAT	TCTATTCGAA	700
TAGCACTAAC	CGATCCGGAT	AATCCTCGAT	GAATTAGTGC	CCAAAAAGAT	750
ATTATTTCTT	ATGTTGATGA	AACAGAGGCA	GCAACTTCAA	CAATTACAAA	800
AAACCAGGAT	GCACAAAATA	ACTGACTCAC	TCAGCAAGCT	AATTTAAGCC	850
CAGCGCCAAA	AGGATTTATT	ATTGCCCCTG	AAAATGGAAG	TGGAGTTGGA	900
ACTGCTGTTA	ATACAATTGC	TGATAAAGGA	ATTCCGATTG	TTGCCTATGA	950
TCGACTAATT	ACTGGATCTG	ATAAATATGA	TTGGTATGTT	TCTTTTGATA	1000
ATGAAAAAGT	TGGTGAATTA	CAAGGTCTTT	CACTTGCTGC	GGGTCTATTA	1050
GGAAAAGAAG	ATGGTGCTTT	TGATTCAATT	GATCAAATGA	ATGAATATCT	1100
AAAATCACAT	ATGCCCCAAG	AGACAATTTC	TTTTTATACA	ATCGCGGGTT	1150
CCCAAGATGA	TAATAATTCC	CAATATTTTT	ATAATGGTGC	AATGAAAGTA	1200
CTTAAAGAAT	TAATGAAAAA	TTCGCAAAAT	AAAATAATTG	ATTTATCTCC	1250
TGAAGGCGAA	AATGCTGTTT	ATGTCCCAGG	ATGAAATTAT	GGAACTGCCG	1300
GTCAAAGAAT	CCAATCTTTT	CTAACAATTA	ACAAAGATCC	AGCAGGTGGT	1350
AATAAAATCA	AAGCTGTTGG	TTCAAAACCA	GCTTCTATTT	TCAAAGGATT	1400
TCTTGCCCCA	AATGATGGAA	TGGCCGAACA	AGCAATCACC	AAATTAAAAC	1450
TTGAAGGGTT	TGATACCCAA	AAAATCTTTG	TAACTCGTCA	AGATTATAAT	1500
GATAAAGCCA	AAACTTTTAT	CAAAGACGGC	GATCAAAATA	TGACAATTTA	1550

TAAACCTGAT	AAAGTTTTAG	GAAAAGTTGC	TGTTGAAGTT	CTTCGGGTTT	1500
TAATTGCAAA	GAAAAATAAA	GCATCTAGAT	CAGAAGTCGA	AAACGAACTA	1550
AAAGCAAAAC	TACCAAATAT	TTCATTTAAA	TATGATAATC	AAACATATAA	1700
AGTACAAGGT	AAAAATATTA	ATACAATTTT	AGTAAGTCCA	GTAATTGTTA	1750
CAAAAGCTAA	TGTTGATAAT	CCTGATGCCT	AA		1762

15. An isolated nucleic acid fragment according to claim 14 encoding a putative protective antigen wherein the antigen is in the 46–48 kD region including the following nucleic acid sequence, mutants, derivatives, recombinants and fragments thereof:

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
ATGAAAAAAA	TGCCACTATA	CCAGAGGAAA	GAGCAGTATA	TAAAATAATT	50
AAAATTACAT	TTTCTTCATT	TGCGCCAGAA	TTTTTAAGAA	TTAGTACATT	100
AAAAAGTAGA	ACAAAAGTTA	TTAATGTAAA	CATTAGCGCA	ATCCTTAAGA	150
AAAAATTAAA	AGTTTTATCT	ATTTTTTTTA	ATCGAAATCC	AACCAGGCAT	200
AAATCTTTGT	CAGTATTTAT	CAAGTCGGTA	TTTTTTCATT	ATTTCTACTA	250
AAATATTATT	TGAATTTGCA	TTTTCCATAA	TCTAAAATTT	TACATTTTTT	300
TATAACAATT	TTTAAAAATT	ACTCTTTAAT	TTATAGTATT	TTTTTATTTT	350
TTAGTCTAAA	TTATAAAATT	ATCTTGAATT	TTATTTGAAT	TTTTATAATT	400
TAGTACTAAA	AAATACAAAT	ATTTTTTCCT	ATTCTAAGAA	AAATTCATTT	450
TTTAAAAAAA	ATTGATTTTT	ATAGTATAAT	TTGTTTGTAT	AATTGAATTA	500
ACTTGATTTG	AAAGGGAACA	AAATGAAAAA	AATGCTTAGA	AAAAAATTCT	550
TGTATTCATC	AGCTATTTAT	GCAACTTCGC	TTGCATCAAT	TATTGCATTT	600
GTTGCAGCAG	GTTGTGGACA	GACAGAATCA	GGTTCAACTT	CTGATTCTAA	650
ACCACAAGCC	GAGACGCTAA	AACATAAAGT	AAGTAATGAT	TCTATTCGAA	700
TAGCACTAAC	CGATCCGGAT	AATCCTCGAT	GAATTAGTGC	CCAAAAAGAT	750
ATTATTTCTT	ATGTTGATGA	AACAGAGGCA	GCAACTTCAA	CAATTACAAA	800
AAACCAGGAT	GCACAAAATA	ACTGACTCAC	TCAGCAAGCT	AATTTAAGCC	850
CAGCGCCAAA	AGGATTTATT	ATTGCCCCTG	AAAATGGAAG	TGGAGTTGGA	900
ACTGCTGTTA	ATACAATTGC	TGATAAAGGA	ATTCCGATTG	TTGCCTATGA	950
TCGACTAATT	ACTGGATCTG	ATAAATATGA	TTGGTATGTT	TCTTTTGATA	1000
ATGAAAAAGT	TGGTGAATTA	CAAGGTCTTT	CACTTGCTGC	GGGTCTATTA	1050
GGAAAAGAAG	ATGGTGCTTT	TGATTCAATT	GATCAAATGA	ATGAATATCT	1100
AAAATCACAT	ATGCCCCAAG	AGACAATTTC	TTTTTATACA	ATCGCGGGTT	1150
CCCAAGATGA	TAATAATTCC	CAATATTTTT	ATAATGGTGC	AATGAAAGTA	1200
CTTAAAGAAT	TAATGAAAAA	TTCGCAAAAT	AAAATAATTG	ATTTATCTCC	1250
TGAAGGCGAA	AATGCTGTTT	ATGTCCCAGG	ATGAAATTAT	GGAACTGCCG	1300
GTCAAAGAAT	CCAATCTTTT	CTAACAATTA	ACAAAGATCC	AGCAGGTGGT	1350
AATAAAATCA	AAGCTGTTGG	TTCAAAACCA	GCTTCTATTT	TCAAAGGATT	1400
TCTTGCCCCA	AATGATGGAA	TGGCCGAACA	AGCAATCACC	AAATTAAAAC	1450
TTGAAGGGTT	TGATACCCAA	AAAATCTTTG	TAACTCGTCA	AGATTATAAT	1500
GATAAAGCCA	AAACTTTTAT	CAAAGACGGC	GATCAAAATA	TGACAATTTA	1550
TAAACCTGAT	AAAGTTTTAG	GAAAAGTTGC	TGTTGAAGTT	CTTCGGGTTT	1600
TAATTGCAAA	GAAAAATAAA	GCATCTAGAT	CAGAAGTCGA	AAACGAACTA	1650
AAAGCAAAAC	TACCAAATAT	TTCATTTAAA	TATGATAATC	AAACATATAA	1700
AGTACAAGGT	AAAAATATTA	ATACAATTTT	AGTAAGTCCA	GTAATTGTTA	1750
CAAAAGCTAA	TGTTGATAAT	CCTGATGCCT	AA		1762

16. A method for producing an antibody against a Mycoplasma including

providing a biological sample taken a short time after an immune animal has been challenged with a Mycoplasma or Mycoplasma extract taken from the infection site or an area of a lesion or an area close to the infection site or lesion;

isolating cells from the biological sample;

culturing cells in vitro in a suitable culture medium; and

harvesting antibodies produced from said cells.

17. A method according to claim 16 wherein the biological sample is taken at a predetermined time after the animal has been challenged with a Mycoplasma, preferably 2 to 7 days after challenge.

18. A method according to claim 16 wherein the culturing of cells in vitro further includes addition of helper factors to the culture, said helper factors selected from the group including cytokines used alone or in combination, including Interleukin 1, 2, 3, 4, 5, 6, 7 and 8, colony stimulating factors, interferons and any other factors that may be shown to have an enhancing effect on specific B cell secretion.

19. A method according to any one of claims 16-18 further including a cell activation step including activating the cells isolated to proliferate and secrete and/or release antibodies

said cell activation step including adding a cell activating agent to the culture medium, said cell activating agent selected from the group including mitogens as herein described and helper factors produced by leukocytes, or their synthetic equivalents or combinations thereof.

20. A method according to any one of claims 16-19 wherein the antibody is in the form of the supernatant harvested from the culture medium.

21. An antibody against a Mycoplasma prepared according to the method of any one of claims 16-20

22. A method of identifying a putative protective antigen associated with a Mycoplasma, preferably Mycoplasma hyopneumoniae, said method including

providing

a sample of a Mycoplasma; and

an antibody probe including at least one antibody against a Mycoplasma;

probing the Mycoplasma sample with the antibody probe to detect at least one antigen; and

isolating the antigen detected.

23. A method of purifying a putative protective antigen associated with a Mycoplasma, preferably Mycoplasma hyopneumoniae, said method including providing

a crude antigen mixture; and

an antibody against a Mycoplasma immobilized on a suitable support;

subjecting the crude antigen mixture to affinity chromatography utilizing the immobilized antibody; and

isolating the purified antigen so formed.

24. A method for preparing a synthetic antigenic polypeptide against Mycoplasma, preferably Mycoplasma hyopneumoniae, which method includes

providing

a cDNA library or genomic library derived from a sample of Mycoplasma; and

an antibody probe including an antibody prepared according to claim 15;

generating synthetic polypeptides from the cDNA library or genomic library;

probing the synthetic polypeptides with the antibody probe; and

isolating the synthetic antigenic polypeptide detected thereby.

25. A method according to claim 24 wherein the antibody probe includes an antibody raised against an antigen against Mycoplasma hyopneumoniae, or related infections, selected from the group of antigens having approximate molecular weights of 110-114, 90-94, 72-75, 60-64, 52-54 and 46-48 kilodaltons (kD), as herein described, mutants, derivatives and fragments thereof.

26. A synthetic putative protective antigen in the 72-75 kD region produced by a method according to claim 24 or 25 having an N-terminal amino acid sequence:

AGXLQKNSLLEEVWYLAL

27. A synthetic putative protective antigen according to claim 26 further including internal amino acid sequences:

AKNFDFAPSIQGYKKIAHEL

NLKPEQILQLLG

LLKAEXNKXIEEINTXLDN

28. A synthetic putative protective antigen in the 60-64 kD region produced by a method according to claim 24 or 25 having an N-terminal amino acid sequence:

MKLAKLLKGFX(N/L)(M/V)IK

ADP(F/I)(R/E)Y(V/A)PQG(Q/A)X(M/N)VG

29. A synthetic putative protective antigen in the 52-54 kD region produced by a method according to claim 24 or 25 having an n-terminal amino acid sequence:

AGXWAKETTKEEKS

30. A synthetic putative protective antigen according to claim 29 further including internal amino acid sequences:

AWVTADGTVN

AIVTADGTVNDNKPNQWVRKY.

31. A synthetic putative protective antigen in the 46-48 kD region produced by a method according to claim 24 or 25 having an N-terminal amino acid sequence:

AGXGQTESGSTSDSKPQAETLKHKV

32. A synthetic putative protective antigen according to claim 31 further including internal amino acid sequences:

TIYKPDKVLGKVAVEVLRVLIAKKNKASR

AEQAITKLKLEGFDTQ

KNSQNKIIDLSPEG

33. A vaccine or veterinary composition including a prophylactically effective amount of at least one putative protective antigen against a Mycoplasma according to any one of claims 1-13.

34. A vaccine or veterinary composition according to claim 33 including a plurality of putative protective antigens selected from antigens having approximate molecular weights of 110-114, 90-94, 72-75, 60-64, 52-54 and 46-48 kilodaltons.

35. A vaccine or veterinary composition including an antibody against a Mycoplasma according to claim 21.

36. A diagnostic kit including a diagnostic antigen or fragment thereof according to any one of claims 1-13 and 26-32.

37. A method for preventing or treating a Mycoplasma infection, which method including administering to an animal a prophylactically or therapeutically effective amount of at least one putative protective antigen according to any one of claims 1-13.

38. An isolated DNA fragment encoding a putative protective antigen against Mycoplasma or related infections, said DNA fragment having a nucleic acid sequence according to Figure 6 or an homologous sequence, and functionally active fragments, mutant, variant or recombinant thereof.

39. A clone including a DNA fragment according to claim 38.

40. A clone according to claim 39 which is clone pC1-2 as hereinbefore described.

41. An amino acid sequence or functional equivalent thereof encoded by the DNA fragment according to claim 38.

42. An amino acid sequence or functional equivalent thereof having the amino acid sequence of Figure 7

43. A putative protective antigen or antibody substantially as hereinbefore described with reference to the examples.

Add A'

and E3